

# Seed Morphology and Anatomy in Salvadoraceae (Brassicales): Systematic and Evolutionary Implications

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To understand the evolution of seed character in Brassicales, we investigated the mature seed morphology and anatomy of four species in all three, poorly understood genera of Salvadoraceae (*Azima*, *Dobera*, and *Salvadora*). The seeds of Salvadoraceae are straight (i.e., not reniform), exarillate, and exalbuminous with a straight embryo. The seed coat is composed of only a testa and lacks a tegmen (including an exotegmen). The thick, multiplicative (except in *A. sarmentosa*) testa has no vascular bundles. The combination of seed and seed coat characteristics supports Salvadoraceae as distinct from the other Brassicales. Evolutionary trends in seed and seed coat (integumentary) characters in Brassicales, particularly the relationships of Salvadoraceae with Bataceae and Koeberliniaceae, are briefly discussed.

Key words: Brassicales, Salvadoraceae, seed, seed coat

Salvadoraceae, a family comprising 11 species in three genera *Azima* Lam. (four species), *Dobera* Juss. (two species), and *Salvadora* Garcin ex L. (five species), are shrubs or small trees of hot, arid regions of Africa (including Madagascar) and southern and Southeast Asia (Kubitzki 2003). The family has been placed in various orders such as Celastrales (Cronquist 1981, 1988, Takhtajan 1986), Oleales (Thorne 1983), Salvadorales (Dahlgren 1989), and Brassicales (Capparales) (Thorne 1992). Morphological and molecular analyses conducted over the past 20 years have shown that Salvadoraceae are placed along with nearly all other glucosinolate-producing families in the order Brassicales (Capparales) (Rodman 1991b, Rodman *et al.* 1993, 1996, 1998, Ronse de Craene & Haston 2006). Within the Brassicales, Salvadoraceae are considered sister to Bataceae, with a clade comprising Salvadoraceae and Bataceae being sister to Koeberliniaceae (Rodman 1991b, Rodman *et al.* 1996) (Fig. 1).

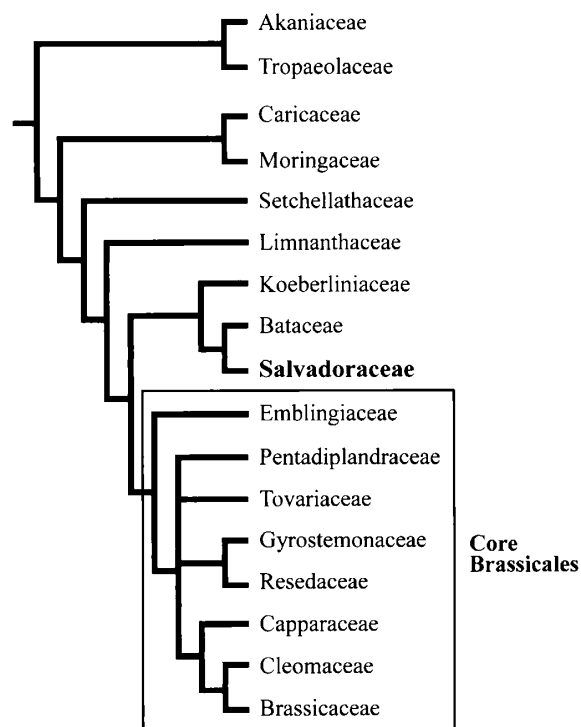


FIG. 1. Phylogenetic tree of Brassicales, indicating position of Salvadoraceae suggested by previous studies. Modified from Stevens (2001 onwards).

During our embryological studies of the glucosinolate-producing families Gyrostemonaceae (Tobe & Raven 1991), Akaniaceae (Tobe & Peng 1990, Tobe & Raven 1995), Setchellanthaceae (Tobe *et al.* 1999), and Koeberliniaceae (Tobe & Raven 2008), we showed that seed characters reflect well the evolutionary relationships in the Brassicales. Anatomical data of the seeds of Salvadoraceae, which have been compared with seeds of other families (Rodman 1991a, 1991b, Tobe & Raven 2008), were based on only one species *Azima tetracantha* Lam. (Maheshwari Devi 1972, Corner 1976). Data from the seeds of additional species and genera of Salvadoraceae are required. Here, we report on the mature seed anatomy of four species from all genera of Salvadoraceae and discuss the evolutionary relationships with other Brassicales, particularly Bataceae and Koeberliniaceae.

## Materials and Methods

Mature seeds of *Azima sarmentosa* Benth. & Hook.f., *A. tetracantha*, *Dobera glabra* A. DC., and *Salvadora persica* Wall. were investigated in this study (for collection data see Table 1). The seeds and seed coats of *A. tetracantha* were described by Maheshwari Devi (1972) and Corner (1976). We included this species in our study because the structural features of the seed coat differ from previous reports and must be revised.

For the anatomical study the seeds from herbarium sheets were soaked in FAA (five parts stock formalin; five parts glacial acetic acid; 90 parts 50% ethanol), then dehydrated through a t-butyl alcohol series and embedded in Paraplast with a melting point of 57–58 °C for microtoming. The paraffin-embedded seeds were softened with a mixture of a 10: 3: 90 glycerol: 10% Aero-

sol OT: water before sectioning (Schmid & Turner 1977). Sections cut 8–10 µm thick were stained with Heidenhain's hematoxylin, safranin and fastgreen FCF, and mounted in Entellan. To examine the entire shape of the individual cells that constitute the mature seed coat, seed tissues were macerated using Jeffrey's method (Johansen 1940). The tissues were then washed in water and stained using Heidenhain's hematoxylin.

For analysis of the seeds and seed coats by scanning electron microscopy, dehydrated seeds were critical-point dried in CO<sub>2</sub> and coated with platinum; observations were made using a JSM-25S (JEOL).

## Results

Mature seeds of the four species of Salvadoraceae examined were collected from berries (*Azima*) and drupes (*Dobera* and *Salvadora*). Examination showed that the seeds are straight, exarillate and exalbuminous with no conspicuous raphe. The seed surface is almost smooth, and the embryo in the mature seed is always straight.

Mature seed coats are usually thick, but lack a tegmen because cells of the inner integument are crushed and disappear during seed development and thus comprise only testal cells in all four species investigated. None of the species has vascular bundles in the testa except in the raphe. Detailed structures of individual species are presented below.

*Azima tetracantha* has seeds that are compressed in the plane of the raphe and are widely elliptic in lateral view, about 4.8–5.2 mm long and 4.5–5.5 mm thick from the raphe to the anti-raphe, and 2–2.2 mm thick from side to side (Fig. 2A, B). The testa is approximately 185–190 µm thick and comprises 9–13 or more cell-layers

TABLE 1. Taxa of Salvadoraceae studied, and voucher information.

| Taxon                    | Voucher   |
|--------------------------|---|
| <i>Azima sarmentosa</i>  | THAILAND. Paknam, south coast of Bangkok. <i>T. Sorensen et al.</i> 2527 (L). |
| <i>A. tetracantha</i>    | SOUTH AFRICA. Albany, Farm "Vaalkranz." <i>R. Bayliss</i> 5158 (MO).          |
| <i>Dobera glabra</i>     | ETHIOPIA. Shoa Prov., Awash National Park. <i>J.W. Ash</i> 1579 (MO).         |
| <i>Salvadora persica</i> | SENEGAL. St. Louis Terr., Sanar. <i>A. Sacques-Georges</i> . 28189 (MO).      |

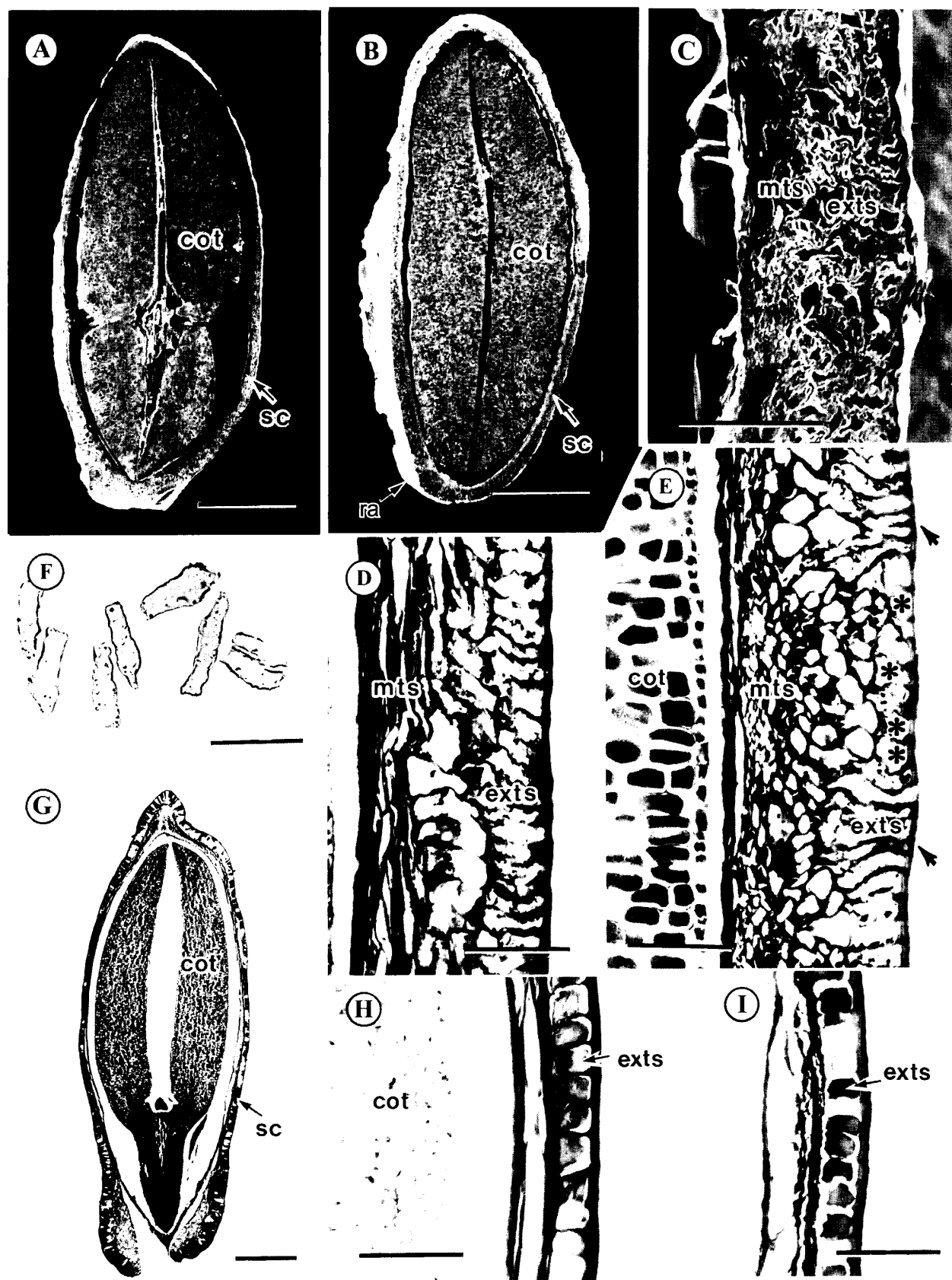


FIG. 2. Seed anatomy of Salvadoraceae. A–F: *Azima tetracantha*. G–I: *Azima sarmentosa*. A, B: Scanning electron micrographs (SEMs), showing longitudinal (LS) and transverse section (TS) of mature seed. C: SEM showing LS of mature seed coat. D, H: LS of mature seed coat. E, I: TS of mature seed coat. F: Cells of exotesta. G: LS of mature seed. Arrow-head in E indicates a group of radially elongate exotestal cells; asterisk in E indicates short or round exotestal cells. cot, cotyledon; exts, exotesta; mts, mesotesta; ra, raphe; sc, seed coat. Bars = 1 mm (A, B), 500  $\mu$ m (G), 100  $\mu$ m (C–F, H, I).

(Fig. 2C–E) as described by Corner (1976, p. 237). All cells of the testa are thin-walled and not lignified. Cells of the exotesta are irregular in shape with a thick cuticular layer on the outer wall. In transverse section (Fig. 2E), the exotesta is composed of both groups of radially elongate cells (e.g., a group of five or six long cells indicated by the arrowhead in Fig. 2E) and groups of much shorter cells. Examination of longitudinal sections through the radially elongate cells (Fig. 2D) shows that the exotesta is a palisade, as described by Corner (1976, p. 237) (see also Fig. 29 in Maheshwari Devi 1972, p. 60). Such radially elongate cells have an end tapering toward the inside of the seed coat (Fig. 2F). Many other exotestal cells are much shorter and even round (see asterisked cells in Fig. 2E), and do not form a palisade. Thus, contrary to Corner's descriptions (1976, p. 237), the exotesta is partially (not evenly) palisadal. Based on analyses of transverse and longitudinal sections, the palisadal and nonpalisadal structures are arranged longitudinally and alternately. According to Corner (1976, p. 237), the exotestal cells are mucilaginous with minute crystals at the outer ends of the radial walls. However, we did not observe either mucilage or minute crystals. Mesotestal cells are varied in shape and size (Fig. 2D, E). Cells on the inner side of the mesotesta are longitudinally elongate (Fig. 2D, E). Endotestal cells, like cells of the tegmen, are probably crushed. No vascular bundles exist in the seed coat.

The seeds of *Azima sarmentosa* are smaller than those of *A. tetracantha*, although they are similar to each other in shape. The seeds of *A. sarmentosa* are compressed in the plane of the raphe and are widely elliptic in lateral view, about 3.8–4.2 mm long and 2.6–3 mm thick from the raphe to the antiraphe, 1–1.4 mm thick from side to side. Unlike *A. tetracantha*, however, *A. sarmentosa* has a thin testa consisting of an exotesta of only a single cell layer (Fig. 2G–I). It is uncertain whether the species had a mesotesta. All endotestal cells and, even if present, all mesotestal cells are crushed and disappear in mature seeds. Cells of the exotesta are cuboidal with a thick cuticular layer on the outer surface (Fig. 2H, I).

*Dobera glabra* has ovoid seeds. They are somewhat compressed in the plane of the raphe and widely elliptic in lateral view, about 10–12 mm long and 7–8 mm thick from the raphe to the antiraphe, 4.3–4.6 mm thick from side to side (Fig. 3A). The testa is approximately 110–130  $\mu\text{m}$  thick and comprises 25–30 cell-layers (Fig. 3B, C). Cells of the exotesta are not clearly distinguished from cells of the mesotesta and endotesta. The mesotesta, which comprises most of the seed coat, appears arenchymatous. Cells of the mesotesta are narrow (about 130–260  $\mu\text{m}$  long), and thick-walled with pits, but they are varied in length and shape (Fig. 3D). Cells of the endotesta are not distinguished from adjacent mesotestal cells.

*Salvadora persica* has broadly ellipsoid seeds that are about 4.8–5.2 mm long and 3.6–4 mm in diameter (Fig. 3E). The testa is approximately 120–140  $\mu\text{m}$  thick (Fig. 3G–I). It comprises an exotesta with a thick cuticle and a thick mesotesta (Fig. 3G–I). The exotestal cells are cuboidal in shape. The mesotesta is about 10–13 cell-layers thick. Cells in the inner third of the mesotesta are collapsed but remain as permanent layers, while those in the outer two-thirds are short (25–70  $\mu\text{m}$  long) and thick-walled with spiral thickenings (Fig. 3F). Cells of the endotesta are not distinguished from adjacent mesotestal cells.

## Discussion

Seed morphology and anatomy of Salvadoraceae has been poorly understood, and most detailed information has been restricted to *Azima tetracantha* (Maheshwari Devi 1972, Corner 1976). In the present study, not only is previous information on *A. tetracantha* revised, but information from one additional species of *Azima* (*A. sarmentosa*) and from the two remaining genera (*Dobera* and *Salvadora*) is provided. Based on all available information, features of the seeds and seed coats of Salvadoraceae can be summarized as follows.

Seeds are straight, exarillate, and exalbuminous with a straight embryo. They are widely elliptic in lateral view and somewhat compressed

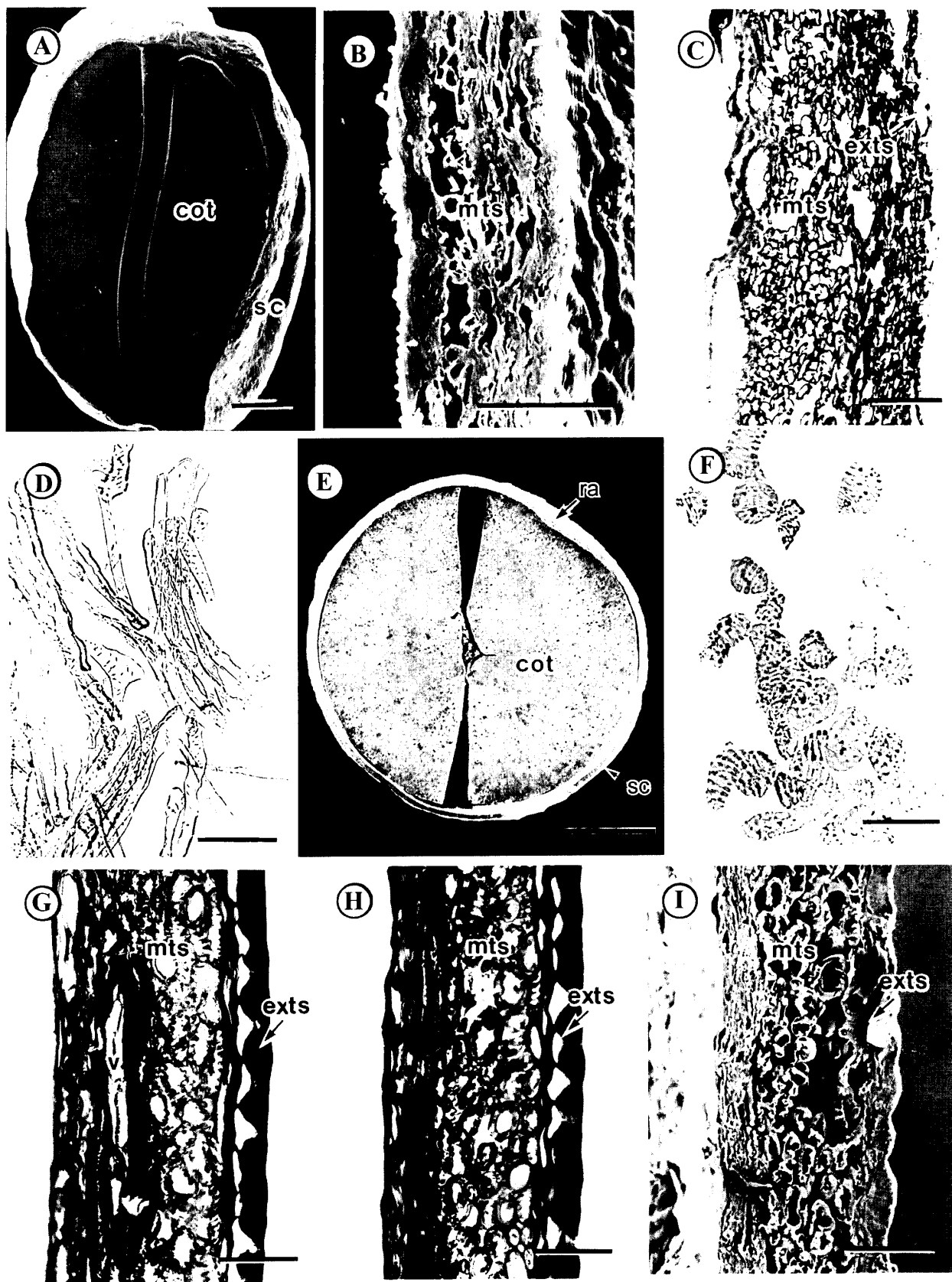


FIG. 3. Seed anatomy of Salvadoraceae. A–D: *Dobera glabra*. E–I: *Salvadora persica*. A, E: Scanning electron micrograph (SEM) showing transverse section (TS) of mature seed. B, I: SEM showing longitudinal section (LS) and TS of mature seed coat. C, H: TSs of mature seed coat. D: Cells of mesotesta. F: Cells in outer two-thirds of mesotesta. G: LS of mature seed coat through raphe. cot, cotyledon; exts, exotesta; mts, mesotesta; ra, raphe; sc, seed coat; v, vascular tissue. Bars = 1 mm (A, E), 100  $\mu$ m (B, D), and 50  $\mu$ m (C, F–I).

(*Azima* and *Dobera*) or ellipsoid (*Salvadora*). Mature seed coats lack a tegmen (including the exotegmen) and comprise only testal cells. The testa is thick with multiple-cell-layers (except in *A. sarmentosa*), and has no vascular bundles. *Azima sarmentosa* has a thin seed coat of only a single cell layer composed only of the exotesta. A comparison of all species examined shows that a testa with multiple cell layers is common to the three genera, whereas a seed coat with a single cell layer is rare in the family. Following typification by Corner (1976) and Schmid (1986), the seed coat of Salvadoraceae is testal, and the testal cells become permanent mechanical layers. Cells of the mesotesta are thin-walled in *Azima tetracantha* but lignified in *Dobera* and *Salvadora*.

In a previous paper (Tobe & Raven 2008), we discussed the evolutionary relationships of Koeberliniaceae, which are considered sister to Bataceae and Salvadoraceae in the Brassicales. Six of the seven characters we selected for the study from more than 50 embryological characters were relevant to seeds and seed coats (or integuments). Table 2 presents the six characters and their respective character states in Salvadoraceae and 16 other brassicalean families. Character-state polarities were determined using Neuradaceae (Malvales) as the outgroup. The character states were mapped on a phylogenetic tree of the Brassicales (modified from Stevens 2001 onwards) using the program MacClade version 3.04 (Maddison & Maddison 2005; Fig. 4A–F). The tree is slightly different from the one used previously, particularly with respect to relationships within the core Brassicales. Although information on those characters is still lacking in several families such as Bataceae, Emblingiaceae, and Pentadiplandraceae, several general tendencies in seed and seed coat evolution are evident, as follows.

Seeds have evolved from being straight to being reniform in shape (Fig. 4A), from possessing to losing vascular bundles in the outer integument (or testa) (Fig. 4B), from having a multiplicative testa to having a nonmultiplicative testa (Fig. 4C), from having a non-multiplicative tegmen to having a multiplicative tegmen (Fig. 4D),

from having a nonfibrous exotegmen to having a fibrous exotegmen (Fig. 4E), and from being exarillate to being arillate (Fig. 4F). Salvadoraceae have a distinct combination of seed and seed coat features not found elsewhere in the Brassicales, i.e., seeds are straight and exarillate, the testa is multiplicative but without vascular bundles, and the tegmen is nonmultiplicative and lacks a fibrous exotegmen (see Fig. 4A–F). In particular, the features of straight seeds without a fibrous exotegmen supports the view that Salvadoraceae are not included in the core Brassicales (Emblingiaceae, Pentadiplandraceae, Tovariaceae, Gyrostemonaceae, Resedaceae, Capparaceae, Cleomaceae, and Brassicaceae) (see Fig. 1).

Considering that Salvadoraceae are sister to Bataceae and in a common clade with Koeberliniaceae, the straight seeds of Salvadoraceae may support, as a reversal from reniform seeds, a sister-group relationship to Bataceae (Tobe & Raven 2008). They may, however, be a plesiomorphy, rather than having been derived as a reversal from reniform seeds (Tobe & Raven 2008). For further discussion of their relationships based on seed characters, more information about Bataceae is needed. We will discuss the morphological relationships between Salvadoraceae and Bataceae more comprehensively in a future paper on Bataceae.

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TABLE 2. Comparison of six selected characters between Salvadoraceae (in boldface) and 16 other families of Brassicales. Order of families according to arrangement from top to bottom in Fig. 4. Character state polarities were determined using Neuradaceae (Malvales) as outgroup. Plesiomorphy is scored as 0, apomorphy as 1. Abbreviations: occ., occasionally; oi, outer integument.

| Characters                           | Akaniaceae   | Tropaeolaceae | Caricaceae         | Moringaceae  | Setchellanthaceae | Limnanthaceae |
|--------------------------------------|--------------|---------------|--------------------|--------------|-------------------|---------------|
| 1 Mature seed straight or reniform ? | Straight (0) | Straight (0)  | Straight (0)       | Straight (0) | Straight (0)      | Straight (0)  |
| 2 Vascular bundles in oi (testa)     | Present (0)  | Present (0)   | Absent (1)         | Present (0)  | ?                 | NA            |
| 3 Testa multiplicative ?             | Yes (1)      | Yes (1)       | Yes (1)            | Yes (1)      | ?                 | NA            |
| 4 Tegmen multiplicative ?            | No (1)       | No (1)        | No (1)             | No (1)       | ?                 | NA            |
| 5 Exotegmen fibrous ?                | No (0)       | ?             | Yes (1)            | No (0)       | No (0)            | NA            |
| 6 Aril                               | Absent (0)   | Absent (0)    | Occ. present (0/1) | Absent (0)   | Absent (0)        | Absent (0)    |

| Characters                           | Koeberliniaceae | Bataceae     | <b>Salvadoraceae</b> | Emblingiaceae | Pentadiplandraceae | Tovariaceae  |
|--------------------------------------|-----------------|--------------|----------------------|---------------|--------------------|--------------|
| 1 Mature seed straight or reniform ? | Reniform (1)    | Straight (0) | Straight (0)         | Reniform (1)  | Reniform (1)       | Reniform (1) |
| 2 Vascular bundles in oi (testa)     | Absent (1)      | ?            | Absent (1)           | ?             | ?                  | Absent (1)   |
| 3 Testa multiplicative ?             | No (0)          | ?            | Yes (1)              | ?             | ?                  | No (0)       |
| 4 Tegmen multiplicative ?            | No (1)          | ?            | No (1)               | ?             | ?                  | No (1)       |
| 5 Exotegmen fibrous ?                | Yes (1)         | ?            | No (0)               | ?             | ?                  | Yes (1)      |
| 6 Aril                               | Present (1)     | ?            | Absent (0)           | Present (1)   | ?                  | Absent (0)   |

| Characters                            | Gyrostemonaceae | Resedaceae   | Capparaceae  | Cleomaceae   | Brassicaceae |
|---------------------------------------|-----------------|--------------|--------------|--------------|--------------|
| 1 Mature seeds straight or reniform ? | Reniform (1)    | Reniform (1) | Reniform (1) | Reniform (1) | Reniform (1) |
| 2 Vascular bundles in oi (testa)      | Absent (1)      | Absent (1)   | Absent (1)   | Absent (1)   | Absent (1)   |
| 3 Testa multiplicative ?              | No (0)          | No (0)       | No (0)       | No (0)       | No (0)       |
| 4 Tegmen multiplicative ?             | No (1)          | No (1)       | Yes (0)      | Yes (0)      | Yes (0)      |
| 5 Exotegmen fibrous ?                 | Yes (1)         | Yes (1)      | Yes (1)      | Yes (1)      | No (0)       |
| 6 Aril                                | Present (1)     | Present (1)  | Absent (0)   | Absent (0)   | Absent (0)   |

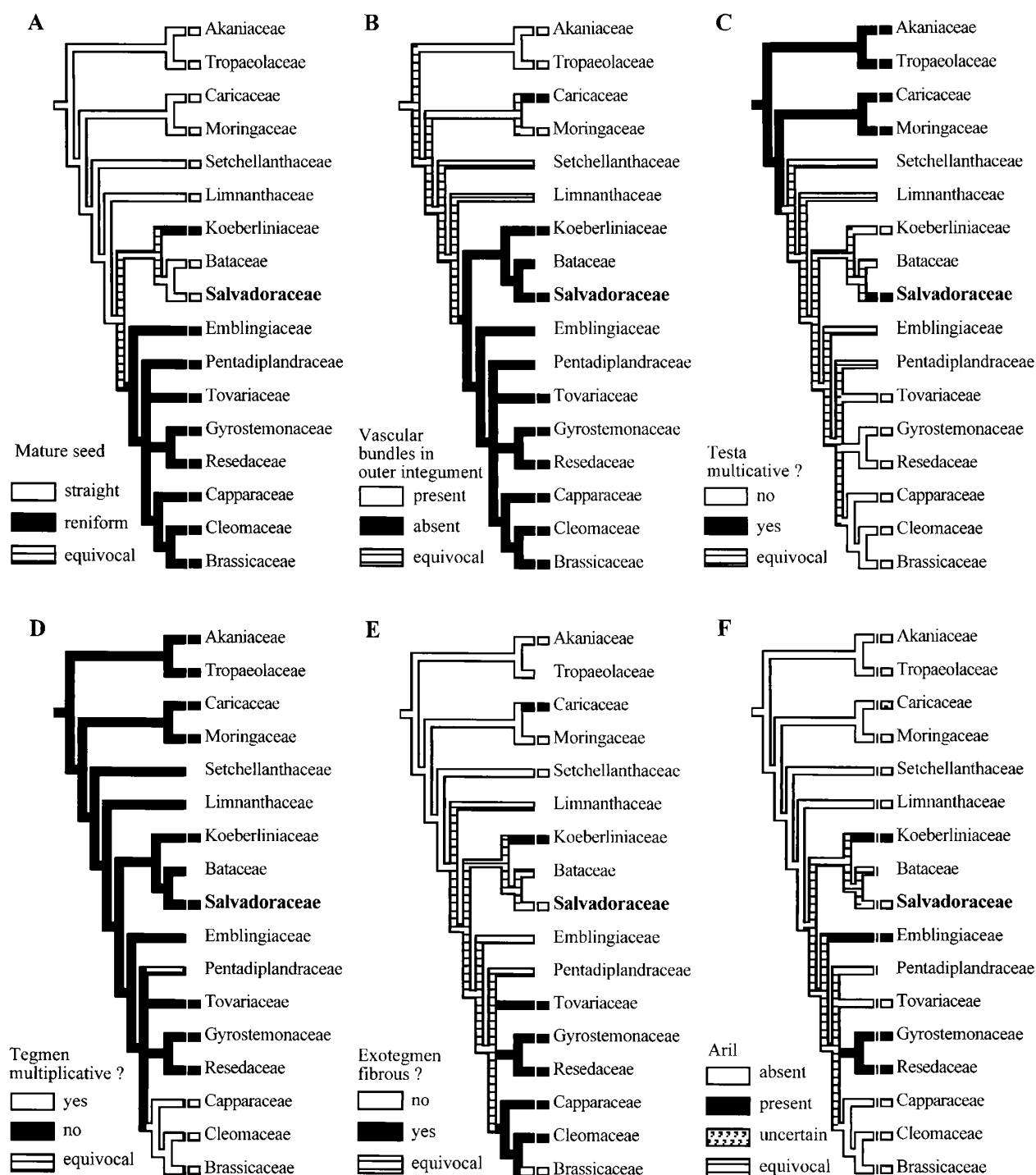


FIG. 4. Distribution (A–F) of character states of six characters (1–6 in Table 2) relevant to seeds and seed coats (or integuments) on phylogenetic tree of Brassicales. Plesiomorphies are indicated in white; apomorphies are indicated in black. A: Seed (straight or reniform). B: Vascular bundles in outer integument (present or absent). C: Testa (nonmultiplicative or multiplicative). D: Tegmen (multiplicative or nonmultiplicative). E: Exotegmen (nonfibrous or fibrous). F: Aril (absent or present).



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